permitted the nitrile symmetric stretch vibration of these UAAs to be unambiguously assigned utilizing the magnitude and direction of the isotopic shift of this vibration. The sensitivity of the nitrile symmetric stretching frequency of each isotopic variant to local environment was measured by individually incorporating the probes into two distinct local environments of sfGFP. The UAAs were also utilized in concert to probe multiple local environments in sfGFP simultaneously to increase the utility of 4-cyano-L-phenylalanine.

3520-Pos Board B675
2-Deuterated Histidine is a Raman Reporter of Histidine's Protonation State, Hydrogen Bonding, and Metal Coordination
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The C-2 proton on the imidazole side chain of the amino acid Histidine (His) can be isotopically exchanged for deuterium under mild reaction conditions, thus creating a unique C-D stretching band that can serve as a site-specific reporter of the environment and role of specific His residues in proteins. This band is a very weak infrared band but is clearly visible in nonresonant Raman spectra. His residues can participate in a number of different interactions unique to the His imidazole ring. Model compound work has shown that the peak frequency and spectral properties of this novel C-D vibrational band is dependent on imidazole protonation state, hydrogen bond donor and acceptor strength, and metal coordination, while salt bridge dependence in the model protein T4 lysozyme is currently being studied. Based on protein work that confirms the model compound protonation dependence, larger and more complex protein systems with multiple, distinct His residues, like superoxide dismutase, are currently targeted to report the environment and interactions of multiple specific His residues within the same protein.

3521-Pos Board B676
The Water Combination Band Reports Solute-Induced Changes in the Dynamic Structure of Bulk Water
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The bend+libration combination band in the infrared spectrum of H2O has important consequences for IR spectra of aqueous samples and for the dynamics of bulk water. The combination band has been shown to respond to solutes and even solvated proteins, complicating baseline subtractions in the region from 1900-2400 cm-1 that is the frequent target of biomolecule-based vibrational probe groups. Due to the less prominent and broad shape of this band, it has been largely overlooked in IR studies of water behavior and dynamics. However, librational motion of water molecules has been shown to be the primary force behind hydrogen bond exchange dynamics, while the bending motion can participate as well. Given the ps-scale of hydrogen bond-forming in liquid water, this band reports directly on these dynamic events through both vibrations, providing a surprisingly clear picture of molecule-scale dynamics in the structure of liquid water. The relationship between the effects of temperature and a range of different solutes on this band is presented here in the first comprehensive study of this band's solute dependence. The effects of denaturants, osmolytes, and solvated salts representing the full extent of the Hofmeister series, among other solutes, will be discussed.

3522-Pos Board B677
Heat Transfer Pathways in Proteins
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Proteins propagate excess thermal energy surprisingly fast and efficient. The theoretical description of thermal transport dynamics in proteins is challenging as it involves both fast quantum vibrations as well as more classical slow conformational changes. Existing protein Molecular Dynamics methods describe these conformational changes well but perform poorly for fast quantum degrees of freedom. Dye labeled Azido-PEG oligomers are experimentally well studied model systems for excess energy transport. Relaxation-assisted two-dimensional infrared (RA 2DIR) spectroscopy has been applied 1) 2) to measure the time-dependent correlation of frequencies belonging to the dye and the Azido group.
Thermal energy is generated at the dye and propagates through the flexible oligomer chain (intramolecular) as well as into the solvent (intermolecular). The oligomer chain undergoes conformational changes on the ps time scales of the transfer process. These slow changes were sampled using Molecular Dynamics trajectories. The resulting structural ensemble was used to map the mode coupling pathways for the fast quantum degrees of freedom. All pairwise mode-mode coupling potentials along pairs of dynamic normal mode vectors were calculated. A molecular heat map was generated using the spatial delocalization of the modes and the inter mode coupling strengths. Molecular heat maps have the potential to extend the theoretical understanding of intramolecular heat transduction pathways in proteins.
1) Lin, Z., Rubtsov, I.V., PNAS, 2012, 105, 5, 1413-1418
2) Lin, Z., Rubtsov, I.V., et al., Phys. Chem. Chem. Phys., 2012, 14, 10445-10454

Bioengineering

3523-Pos Board B678
Rational Design of a Zinc Phthalocyanine Binding Protein
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Phthalocyanines have long been used as primary donor molecules in synthetic light-powered devices due to their superior properties when compared to natural light activated molecules such as chlorophylls. their use in biological contexts, however, has been severely restricted due to their high degree of self-association, and its attendant photoquenching, in aqueous environments. To this end we report the rational redesign of a de novo four helix bundle di-heme binding protein into a heme and Zinc phthalocyanine dyad. The stepwise design pathway included the sulfonation of the phthalocyanine cofactor to destabilize stacking interactions, the creation of a single chain helical bundle protein in order to enable the creation of asymmetric binding sites and the removal of a disulfide bond to increase protein flexibility. The final design tightly binds one heme and one zinc phthalocyanine as a monomer in ''splendid isolation''. Singular binding of Zinc Phthalocyanine was verified by absorption, fluorescence and magnetic circular dichroism spectroscopies.

3524-Pos Board B679
Photo-Regulation of Small G Protein Ras Mutants using Photochromic Molecule
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Ras is one of small G-proteins known as a molecular switch mediating cellular signalling. Switching on state of Ras is induced by exchange of bound GDP for GTP and off state is by hydrolysis of GTP to GDP. Interestingly, the core nucleotide-binding motif of Ras is considerably conserved with the ATP driven motor proteins, myosin and kinesin. Therefore, it is believed that these biomolecular machines share common molecular mechanism utilizing nucleotide hydrolysis cycle. Previously, we have incorporated photochromic molecules, 4-phenylazophenyl maleimide (PAM), into the functional site of kinesin as a photo-switching device and succeeded to regulate kinesin ATPase activities reversibly upon visible light (VIS) and ultra-violet (UV) light irradiation. Therefore, it is expected that Ras can be also regulated by the similar method using photochromic molecules.
In this study, we performed basic study to control the function of Ras reversibly using photochromic molecules upon VIS and UV light irradiations. First, in order to monitor the exchange of bound GDP for GTP, we synthesized a new fluorescent GTP analogue, NBD-GTP, and GDP analogue, NBD-GDP, which change their fluorescent intensity along the formation of Ras-GTP, Ras-GDP-Pi and Ras-GDP states. And the GTPase activity of Ras was monitored by the quantitative analysis of GTP and GDP in the active site of Ras using reverse phase column chromatography on HPLC. We have designed three kinds of Ras mutants K5C, I36C, and Y64C. The mutants were prepared using *E.coli.*expression system and modified with PAM. It was suggested that the GTPase activities of Ras mutants modified with PAM were reversibly alternated upon ultra-violet and visible light irradiation.

3525-Pos Board B680
A Lattice Model of Cross-Linked Polymeric Materials: The Role of Frustration
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The diversity of properties of cross-linked polymeric materials cannot be explained by the constituent polymer chains alone but is attributed in part to their cross-linking. We propose a three-dimensional lattice model of bulk, polymeric materials that consist of stiff, cross-linked chains. Each chain has a randomly distributed, fixed number of binding sites that are capable of forming cross-links, which we call "active binding sites" (ABSs). The number of ABSs per unit length of chain, their interaction strength, and their distribution are the key parameters by which variability is achieved in representative materials. Inevitably, systems with a random distribution of ABSs retain unsatisfied bonds. We refer to these as "frustrated" systems. Numerically, an interfacial crossover is observed from an ordered state at low temperature to a disordered state at high temperature; the crossover scale depends on ABS density. Randomness